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PRINCIPAL INVESTIGATOR: David S. K. Magnuson, PhD.

CONTRACTING ORGANIZATION:
University of Louisville
Louisville, KY 40208-1838

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14. ABSTRACT Essentially all spinal cord injured patients receive stretching therapies beginning within the first few weeks post-injury. Despite this fact, almost nothing is known about how stretching might influence the neural circuitry in the spinal cord that is responsible for controlling the motor and locomotor activities of the legs. Recently, while studying activity-based rehabilitation in a rat model of spinal cord injury, we observed that stretching actually worsened locomotor recovery. The goal of this project is to investigate how the timing and intensity of a stretch-based therapy influences locomotor recovery after moderate and severe spinal cord injuries. In this, the first year of this award, we have found that stretching negatively influences locomotor function in animals with both acute (within days) and chronic (after 3 months) spinal cord injuries. We have also determined that stretching for short periods of time (4-5 weeks) allows substantial recovery to occur once stretching is stopped, and both acute and chronic animals show a similar time course of recovery. Finally, in very preliminary studies, we have found that the torque being applied during stretching of the rat hindlimb is roughly similar to that applied to human lower extremities relative to body weight.					
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Annual Report:

SC110169 – “Directing Spinal Cord Plasticity: The Impact of Stretch Therapy on Functional Recovery after Spinal Cord Injury”.

Principle Investigator: David S. K. Magnuson, PhD. University of Louisville.

Introduction:

This research focuses on the impact of stretching (physical therapy maneuvers involving force or torque applied to specific muscle groups) on functional recovery after spinal cord injury in a rat model. We have undertaken these studies because of an observation we made a few years ago during a study where the hindlimbs of rats with spinal cord injuries were being immobilized in a wheelchair. We found that immobilization dramatically influenced locomotor recovery, presumably, by reducing the sensory input associated with movement (Caudle et al., 2011). In that study we employed a stretching procedure designed to prevent reductions in joint range-of-motion. The stretching didn't prevent contractures, but it did have a negative impact on locomotor recovery. Thus, in the current study, specific Aim (SA) 1 focuses on the timing of stretching relative to the injury and whether or not there is a window of susceptibility to a stretching-based therapy. SA2 focuses on the pattern and forces of the actual stretching protocol and if the negative influence of stretching is due primarily to the length of each maneuver or to the forces applied during stretch. An overarching goal in the project is to develop a computer model of the stretching to allow direct comparison of rat and human.

Body:

In this, the first year of this project, we have accomplished substantial components of Tasks 1 and 3 as described in the original Statement of Work. This has involved aspects of Specific Aims 1 and 2 as follows.

Forces Applied During Stretching.

The specific experiments completed during the first quarter of this year focused on measuring the forces being applied to hindlimb muscle groups in both uninjured and injured rats. This involved the use of FSRs (Force Sensitive Resistors) and kinematics (quantification of joint angle and/or limb position) during stretch maneuvers and developing a custom-built data acquisition system and program. Mr. Erik Seibt was the primary individual involved (a Bioengineering Masters student) along with Ms. Anastasia Prokopenko (a PhD student). They recorded both kinematics and forces during stretching of uninjured rats (under isoflurane anesthesia) and of these same rats following injury (unanesthetized). The data shows first of all that the FSR-based force glove, data acquisition system and software work very well. The results demonstrate that forces about the ankle of the rat during therapeutic stretching are quite similar over time, both in uninjured and spinal cord injured rats with some modest drop in forces applied acutely post-injury. This suggests that the forces required to reach end range-of-motion (ROM) are less in the first few weeks post-injury. The results also show that the torques required to reach end-ROM for ankle flexion and extension range from 1.5 N-cm immediately after the injury to 2.5 N-cm pre-injury and again at 5 weeks post-injury. One of the primary goals of this project is to compare the stretching applied to rats with published data for human subject. Published data shows that ankle stretches in human subjects can require torques in the range of 2 to 10 N-m. If we simply scale this to the rat based on body weight the equivalent torques would be 0.8 to 4.2 N-cm, a range that includes our observed torques. This is an outstanding initial finding that suggests the stretching being applied to rats is quantitatively similar to that being applied clinically to human patients. Our data also shows that two different therapists applied similar torques for most, but not all stretching sessions. The two graphs show calculated torques for the TS and TA muscle groups (ankle extensors and flexors, respectively).

Figure 1

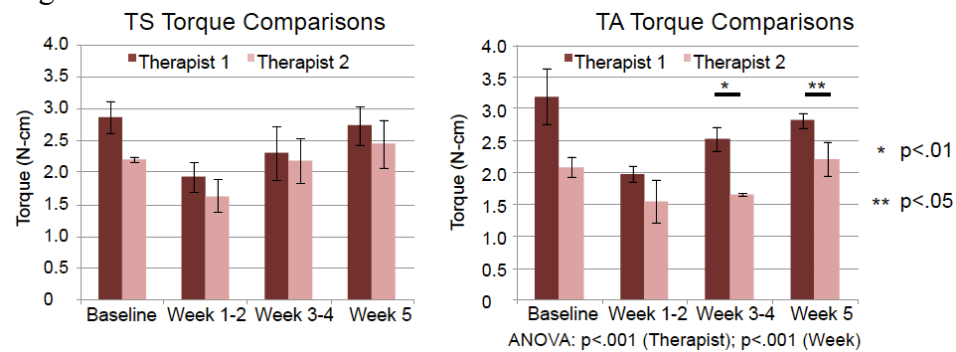


Figure 1 Legend. Figure 1 shows the calculated torques for ankle flexion stretches (left) and ankle extension stretches (right) at baseline (anesthetized) and post-injury. Interestingly, these data show that the muscle tone drops acutely post-injury, even compared to the baseline measurements made under isoflurane anesthesia.

Stretching of Acutely Injured Animals:

For SA 1, a major study was initiated in mid-June. This study is designed to examine the influence of our stretching protocol on animals with moderately-severe contusion injuries at the T9 level of the spinal cord. This is a more severe injury that we have investigated so far, and is a very clinically relevant severity because most of the animals will not recovery coordinated stepping. This study is large, involving 2 groups of 10 animals, plus 5 controls. One group ($n=10$, acute) will receive the daily stretching protocol starting 1 week post-injury and continuing for 5 weeks. The chronic group will be stretched daily from week 12 to week 16. For this study we used 7 different “therapists” (students or technicians) who performed the daily stretching protocol. As is often the case in biomedical research, we encountered an unexpected issue that, at first, seemed to be a problem, but in fact has turned out to be very interesting. At week 2 and 3 post-injury, these animals developed higher than expected muscle tone (due to the more severe injury) and the therapists were unsure about how to perform the stretches. We sought the advice of Mr. Darryn Atkinson and Ms. Carie Tolfo, two very experienced physical therapists from the Frazier Rehab Institute. They have over 15 years of experience (between them) performing stretch-based therapy on spinal cord injured patients. They observed and participated in several stretching sessions and instructed the therapists to “stretch through the increased tone using constant forces that were sufficient to reach a normal end-range-of-motion over a period of 10-15 seconds and to maintain that position for the desired 1 minute”. This strategy was adopted starting at week 4 and, as you can see from figure 2 below, it had a dramatic effect on the function of the stretched animals. I will remind you that the BBB scale being used here is a visual scale that assesses hindlimb movement during overground stepping. Normal is 21; stepping with weight support but without coordination is 11-12, plantar paw placement is 8-9. Scores under 8 represent various non-plantar limb movements that do not contribute to locomotion (sweeping). As you can see, the stretched animals showed a dramatic drop over weeks 4 and 5. We have now followed the progression of the stretched animals and found that they recover to control levels over a period of two-three weeks. Thus, this study has determined that stretching, initiated acutely, has a negative impact on animals with moderately-severe injuries. These observations have very significant clinical implications.

Figure 2.

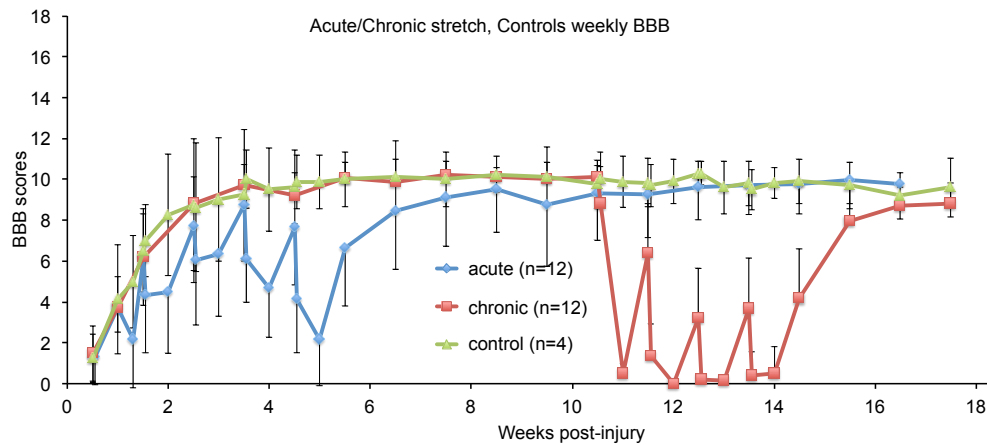


Figure 2 Legend. This graph shows the BBB scores for the acute and chronic stretch groups that were stretched daily, M-F using our 30 minute stretching protocol compared to the control, unstretched group of animals. The error bars are standard deviation. Final statistical analysis has not yet been done, but a basic ANOVA and t-test shows that BBB scores are different at week 4 and 5 for the acute group and for weeks 11 through 14 for the chronic group, compared to controls. BBB scores were done on Monday mornings (before stretching), Monday afternoons (after stretching) and on Friday afternoons after the five stretching sessions for that week. It is important to note that a drop from 10 to 3 or 4 on the BBB scale is functionally devastating with the animals going from “stepping with weight support but without coordination” to “movement of 2 or 3 joints without sweeping”.

Stretching of Chronically Injured Animals.

We are just completing the chronic part of the major study, described above, that was initiated in mid-June. This study has so far successfully addressed the goals of examining the influence of post-injury timing on how our stretching protocol influences functional recovery following moderately-severe contusion injuries at the T9 level of the spinal cord. As discussed in my previous report, this study is being managed by Ms. Anastasia Prokopenko, a new Physiology graduate student in the lab. She took over the position of primary driver when Dr. Krista Caudle left the lab to pursue postdoctoral training. We recently submitted a letter requesting formal exchange of Ms. Prokopenko for Dr. Caudle on the Grant. Anastasia has done a fantastic job of the study so far. As you can see from Figure 2, the acute animals recovered substantially once stretching was stopped at week 5. Their locomotor function was not different from the control group by week 7. This is a very interesting observation that we will follow-up on in future studies. Also in Figure 1 you can see that the stretching protocol had a dramatic (one could say devastating) effect on the locomotor function of chronically injured animals. In rodent studies it is generally accepted that 8-10 weeks post-injury constitutes a “chronic” phase, and we waited an additional few weeks. Several key points should be made about the chronic animals: 1.

Therapists reported that the animals were “very flaccid” when they started stretching (over the first 3-4 days of stretching), suggesting that these animals had muscle tone that was lower than expected (compared to the acute animals). 2. By the end of the first week of chronic stretching, the muscle tone (resistance to stretch) had increased noticeably. 3. Therapists also reported that hindlimb muscle groups were losing mass by the end of the second week of stretching. Given that the animals had BBB scores of 0 (showing no hindlimb movement at all during overground stepping), the muscle wasting is not surprising, but is an interesting finding nonetheless. 5. Once stretching ceased, the profile of recovery for the chronic animals was very similar to that of the acute animals, and there was no difference in function between the stretched and control groups 2-3 weeks later. The outcomes of this experiment are outstanding because they are so

unequivocal. The effect of stretching after spinal cord injury is just as devastating for chronic animals as it is for acute animals and, once stretching ceases after 4 weeks, the changes to the spinal cord circuitry are not lasting, at least for animals with fairly severe injuries.

Key Research Accomplishments.

The results described above are very significant and carry huge implications for the clinic and care of patients with spinal cord injury. They suggest the following:

1. The forces being applied during stretching of the rat are roughly similar to those being applied to humans clinically, based on a body-weight comparison.
2. That daily stretching, whether applied in the acute or chronic phase, can be devastating to motor function, and in the light of our previous work, if stretching is continued for 8 weeks or more, the deficits may be long-lasting.
3. That deficits resulting from both acute and chronic stretching can be viewed as temporary if the stretching is only done for a few weeks. This finding has to be viewed with the caveat that rats are more active than people (post-injury) and that this activity may be what allows them to recover after several weeks of stretching.

These findings are extremely significant and will be strengthened as all the data is analyzed and the histological data is acquired and analyzed which is necessary to show that the injuries were similar and either were or were not influenced by acute stretching.

Reportable Outcomes:

An abstract has been submitted to, and accepted for presentation at the International Symposium on Neural Regeneration that will be held at the Asilomar Conference Center in Monterey, CA in December, 2013. The abstract is entitled “Muscle stretch reduces locomotor function after a spinal cord injury: Acute and chronic time points. It will be presented as a poster, and possibly as a short talk at the symposium by Anastasia Prokopenko. The abstract is attached as an appendix.

Conclusion:

Our results so far are extremely important because they validate what we found previously (Caudle et al., 2011) and extend the findings to more clinically relevant severe and chronic spinal cord injuries. Furthermore, they show that the forces we are using while stretching the rats are, at least based on body weight, comparable to what is routinely applied in the clinical setting. Thus, there is solid rationale for proceeding with the remaining experiments as described in the original proposal. None of our results are pointing towards the development of a product, but will lead to the suggestion that our current stretching practices in the clinic will need to change. Our remaining experiments will help to illustrate how those practices should change.

References:

Caudle KL, Brown EH, Shum-Siu A, Burke DA, Magnuson TSG, Voor MJ, Magnuson DSK. Hindlimb immobilization in a wheelchair alters functional recovery following contusive spinal cord injury in the adult rat. *Neurorehab and Neural Repair*. 25: 729-39, 2011. PMID: 20302862

MUSCLE STRETCH REDUCES LOCOMOTOR FUNCTION AFTER A SPINAL CORD INJURY: ACUTE AND CHRONIC TIME POINTS

A.V. Prokopenko, A. Shum-Siu, G.N. Wainwright, E. Seibt and D.S.K. Magnuson.
Departments of Physiology and Biophysics, Neurological Surgery, Bioengineering and Anatomical Sciences and Neurobiology. University of Louisville, Louisville, KY

After spinal cord injury (SCI) patients commonly develop spasticity and contractures as secondary complications of “upper motor neuron” lesions. Physical therapists use stretching maneuvers to maintain extensibility of soft tissues and to manage spasticity. Previous studies in our lab showed that stretching negatively effects locomotor recovery in rats with mild thoracic SCIs. The present study was undertaken to extend this observation to a more clinically relevant moderately-severe contusion, to compare stretching at acute and chronic time points and to determine if stretching influences in-cage activity. Female SD rats with 25g-cm T10 contusion injuries received our standard 24 minute stretch protocol, daily for 5 weeks starting 4 days (acute) or daily for 4 weeks starting 12 weeks (chronic) post-injury. Deficits in locomotion were evident in the acute animals after only 5 days of stretching. The ‘therapists’ noted increased stiffness or tone by week 3 that reduced the range-of-motion (ROM) of all three joints. For weeks 4 and 5, it was decided that the therapists should stretch through the tone, using increased pressure to achieve a normal end ROM. This mode of stretching resulted in even greater impairments in locomotion. In the chronic group dramatic drops in locomotor function were also observed after only 5 days, and most animals had BBB scores of 0-3 for weeks 2, 3 and 4 of stretching. Importantly, overnight activity did not differ between groups at any time point, and locomotor function recovered to control levels for both stretch groups within 3 weeks once daily stretching ceased. Our findings show that stretching is detrimental to locomotor recovery for animals with clinically relevant moderately-severe contusion SCIs, that the deficits are not due to reduced in-cage activity and that they do not persist if stretching ceases after 5 weeks.

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